Difference in rates of net portal absorption between crystalline and protein-bound lysine and threonine in growing pigs fed once daily

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Difference in rates of net portal absorption between crystalline and protein-bound lysine and threonine in growing pigs fed once daily[1]

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ABSTRACT: Net portal absorption of AA during the 6-h postprandial period was measured in eight gilts (48.5 ± 1.6 kg BW) in a crossover design. The pigs had chronic catheters placed in the portal vein, carotid artery, and ileal vein, and were trained to consume 1.2 kg of a standard grower diet once daily. Blood samples were taken every 30 min for 4 h and then hourly until 6 h after feeding. The first set of blood samples was taken after pigs were fed a meal of the test 16% CP corn–soybean meal diet (16% CP) or the test 12% CP corn–soybean meal diet supplemented with crystalline lysine, threonine, and tryptophan (12% CP + AA) to equal the three AA levels in the 16% CP diet. Pigs were then fed the standard diet for 2 d. Following that, blood samples were again taken after the pigs were fed a meal of the test diet that was not given to them at the first sampling period. Net portal AA absorption was calculated by multiplying porto-arterial plasma AA concentration difference by portal vein plasma flow rate (**PVPF**), estimated by an indicator-dilution technique employing *p*-aminohippuric acid as the indicator infused into the ileal vein. Plasma concentrations of lysine and threonine of pigs were affected by the diet × time interaction ($P < 0.01$). Portal and arterial plasma lysine and threonine concentrations in pigs attained the maximal level by 1 h postprandial when the 12% CP + AA diet was fed, but reached the peak level at 2.5 h postprandial when the 16% CP diet was given. The PVPF of pigs over the 6 h postprandial was less ($P < 0.01$) when the 12% CP + AA diet was given than when the 16% CP diet was fed. Net portal absorptions of lysine and threonine also were affected ($P < 0.05$) by time × diet interaction. The peak portal absorption of both lysine and threonine in pigs appeared at 0.5 h postprandial when the 12% CP + AA diet was given, but at 2.5 h postprandial with the feeding of the 16% CP diet. The early appearance of peak portal absorption of lysine and threonine from feeding the 12% CP + AA compared with the 16% CP diet indicates that crystalline lysine and threonine are absorbed more rapidly than protein-bound lysine and threonine in pigs fed once daily.

Key Words: Crystalline Amino Acids, Lysine, Pigs, Portal Absorption, Protein-Bound Amino Acids, Threonine

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Introduction

Reducing dietary protein level and supplementing with certain crystalline AA decreases nitrogen excretion and environmental pollution (NRC, 1998). The efficiency of some crystalline AA utilization by pigs, however, is affected by feeding frequency. When diets supplemented with crystalline lysine were fed to pigs once daily rather than six or three times a day, a lower response was produced for carcass gain, feed conversion, dressing percent, or N retention (Batterham and Murison, 1981; Cook et al., 1983). Inefficient utilization of crystalline threonine by pigs with once-daily feeding had also been reported (Cole, 1992). Because pigs are generally allowed ad libitum access to feed, inefficient utilization of crystalline AA may not be a practical problem. However, it could still be an issue in situations where pigs are fed once daily, such as gestating sows or feed-restricted pigs used in many research studies.

Batterham and Bayley (1989) observed greater oxidation of ^{14}C-labeled phenylalanine by pigs fed once daily a diet supplemented with crystalline lysine than by those given a diet containing only protein-bound lysine. It was hypothesized that a difference in the rate of absorption between crystalline lysine and protein-bound lysine existed in pigs fed once daily, and that an imbalance of AA occurred at the sites of protein synthesis due to more rapid absorption of crystalline

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Table 1. Composition of standard diet, as-fed basis

Item	%
Ingredient	
Corn, yellow, grain	76.5
Solvent-extracted soybean meal	19.6
Dicalcium phosphate	2.4
Ground limestone	0.5
Iodized NaCl[a]	0.4
Trace mineral premix[b]	0.2
Vitamin premix[c]	0.2
Choline chloride[d]	0.2
Calculated	
CP	16.0
Lysine, total	0.75
Threonine, total	0.66
Tryptophan, total	0.16
Ca	0.99
P, total	0.80
Analyzed	
CP	16.11
Lysine, total	0.74
Threonine, total	0.67
Tryptophan, total	0.15

[a]Supplied 0.28 mg of iodine per kilogram of diet.
[b]Supplied the following in milligrams per kilogram of diet: Fe (as ferrous sulfate heptahydrate), 160; Cu (as cupric oxide), 10; Mn (as manganese oxide), 20; Zn (as zinc oxide), 100; Se (as sodium selenite), 0.1; and $CaCO_3$ was used as a carrier.
[c]Supplied the following per kilogram of diet: retinyl acetate, 5,280 IU; cholecalciferol, 704 IU; DL-alpha-tocopheryl acetate, 70.4 IU; menadione sodium bisulfite complex, 3.5 mg; vitamin B_{12}, 26.4 μg; riboflavin, 5.3 mg; niacin, 28.2 mg; d-pantothenic acid, 21.1 mg; d-biotin, 88 μg; and thiamin, 2.2 mg.
[d]Supplied 868 mg of choline per kilogram of diet.

lysine than protein-bound lysine. However, direct measurement of the absorption of lysine by pigs was not conducted.

The objectives of the present study were to directly measure the absorption of AA into the portal vein and to demonstrate the difference in absorption rates between crystalline and protein-bound lysine and threonine in growing pigs fed once daily a diet containing protein-bound lysine and threonine or both crystalline and protein-bound lysine and threonine. Partial results of this study were reported previously (Yen et al., 1991).

Materials and Methods

The experiment was approved by the Animal Care and Use Committee of the U.S. Meat Animal Research Center (**MARC**). A total of eight crossbred (¼ Chester White, ¼ Landrace, ¼ Large White, ¼ Yorkshire) growing gilts (36.4 ± 1.2 kg initial BW) were used. Pigs were trained to consume within a 30-min period once daily 1.2 kg of MARC standard grower diet mixed with 1.2 L of water. The MARC standard diet (Table 1) was a fortified corn-soybean meal diet formulated to meet or exceed all NRC (1988) nutrient requirements. The use of 1988 rather than 1998 NRC requirements was because the study was conducted before the publication of 1998 NRC requirements. The standard diet was analyzed to have 16.1% CP, 0.74% total lysine, 0.67% total threonine, and 0.15% total tryptophan (as-fed basis). Pigs were placed into rectangular metabolism cages at 0930 to receive their daily feed. The metabolism cages had adjustable sides and back (81 cm in height) and a 46 cm × 122 cm woven-wire floor, which was 81 cm above the ground. After feeding, the pigs were put back in their 1.2 m × 1.2 m pens, which had a nipple waterer to provide water at all times. The temperature of the rooms housing the pigs was maintained at 21°C with 24-h lighting.

When pigs weighed 42.1 ± 1.4 kg, chronic catheters were surgically placed in the hepatic portal vein, carotid artery, and ileal vein. Detailed surgical procedures and construction of the catheters have been described previously (Yen and Killefer, 1987). A pig was considered fully recovered when it had regained its preoperation appetite.

After they had maintained their preoperation appetite for at least 7 d, the eight pigs were grouped into four pairs. Each pair of pigs was used in a randomized 2 × 2 Latin square (simple cross-over design). The paired pigs were weighed 6 h postprandially at 1530 and placed into rectangular metabolism cages each day. On the following day at 0800, the ileal vein catheter of each pig was connected to a 2-m Tygon microbore tubing (0.75 mm i.d.; 2.29 mm o.d.). The distal end of the Tygon tubing was then connected to a sterile disposable filter assembly (0.2-μm pore size) attached to a 50-mL syringe of the infusion-withdraw pump. The pig was primed at 0825 at a rate of 3.82 mL/min for 5 min with a 0.9% saline solution containing 1.0% *p*-aminohippuric acid (**PAH**), pH = 7.45. After priming, the PAH solution was infused constantly at a rate of 0.788 mL/min for 7 h. The portal vein and carotid artery catheters were each connected to 1.2 m of heparin complex-treated polyurethane tubing (1.68 mm i.d.; 2.41 mm o.d.). The distal end of the tubes was then connected to a multiple-channel peristaltic pump and a three-way stopcock for blood sampling and for flushing the tubing with heparinized saline solution. The specifications and sources of the tubing, filter assembly, infusion pump, and peristaltic pump have been described previously (Yen et al., 1989).

For each of four Latin squares, one pig was randomly assigned to receive a test 16% CP corn–soybean meal diet (**16% CP**). The other pig was assigned to receive a 12% CP corn–soybean meal diet supplemented with crystalline lysine, threonine, and tryptophan (**12% CP + AA**). Crystalline AA additions to the 12% CP + AA diet were based on the analyzed AA concentrations in the corn and the dehulled soybean meal (as-fed basis). The two test diets were calculated to contain equal amounts of total lysine, total threonine, and total tryptophan (Table 2). The two test diets were prepared at the University of Illinois and analyzed for CP (N × 6.25) and AA contents with procedures similar to those reported previously (Kerr and Easter, 1995). The analyzed values of CP and AA of the two test diets are

Table 2. Composition of test diets, as-fed basis

Item	Dietary treatment	
	16% CP	12% CP + AA
Ingredient, %		
Corn, yellow, grain	76.90	86.16
Dehulled solvent-extracted soybean meal	20.65	10.80
Dicalcium phosphate	0.91	0.97
Ground limestone	1.09	1.03
Trace mineral salt[a]	0.35	0.35
Vitamin premix[b]	0.10	0.10
L-Lysine·HCl	—	0.35
L-Threonine	—	0.16
DL-Tryptophan	—	0.08
Calculated composition, %[c]		
CP	16.00	12.00
Ca	0.63	0.60
P, total	0.53	0.50
Metabolizable energy, Mcal/kg	3.40	3.45
Lysine, total	0.83	0.83
Threonine, total	0.67	0.67
Tryptophan, total	0.19	0.19
Methionine, total	0.29	0.24
Analyzed composition, %[d]		
CP	15.70	12.30
Lysine, total	0.82	0.83
Threonine, total	0.62	0.63
Tryptophan, total	0.17	0.16
Arginine, total	1.04	0.74
Histidine, total	0.45	0.38
Isoleucine, total	0.69	0.52
Leucine, total	1.57	1.32
Phenylalanine, total	0.81	0.63
Valine, total	0.82	0.62

[a]Supplied the following per kilogram of diet: Se (as sodium selenite), 0.1 mg; I (as ethylenediamine dihydroiodide), 0.35 mg; Cu (as cupric oxide), 8 mg; Mn (as manganese oxide), 20 mg; Fe (as ferrous sulfate haptohydrate), 90 mg; Zn (as zinc oxide), 100 mg; NaCl, 2.87 g.

[b]Supplied the following per kilogram of diet: retinyl acetate, 3,300 IU; cholecalciferol, 330 IU; DL-α-tocopheryl acetate, 22 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 2.2 mg; d-calcium pantothenate, 6.1 mg; niacin, 16.6 mg; choline chloride 165.4 mg; vitamin B_{12}, 0.02 mg.

[c]Calculated values were based on analyses of feed ingredients for CP and CP:AA ratios for estimates of AA concentration. The CP analyses were 7.8% for the corn and 48.5% for the soybean meal.

[d]Methionine levels were not analyzed.

presented in Table 2. Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis.

At 0930, 1.2 kg of each test diet was mixed with 1.2 L of water and given to the pigs. Portal and arterial blood samples were obtained simultaneously by activating the multiple-channel peristaltic pump before feeding, once every 30 min during the first 4 h postprandial and hourly from 5 to 6 h postprandial. Blood samples (5 mL each) were drawn into heparinized syringes and stored on ice. Within 15 min of collection, the blood was centrifuged at 4°C and 3,300 × *g* for 10 min to separate plasma from cells. An aliquot of plasma was refrigerated and assayed within 12 h for PAH concentration as described previously (Yen and Killefer, 1987). Another aliquot was stored at –20°C until analyzed for AA concentration. For AA analysis, the frozen plasma samples were thawed at 4°C and deproteinized using 30 mg of sulfosalicylic acid per milliliter of plasma (Perry and Hansen, 1969). The AA concentration of deproteinized plasma was determined by ion-exchange chromatography with an automated AA analyzer employing a three-buffer, single-column method (Beckman Amino Acid Analyzer, model 121MB with model 126 Data System, Beckman Instruments, Inc., Palo Alto, CA).

After the first sequence of blood samplings, the pigs were returned to their home pens and fed the standard diet once daily for 2 d. Following that, they were then weighed 6 h postprandially and again placed into rectangular metabolism cages. On the following day, the pigs were fed at 0930 the test diet that they were not provided during the first sampling period, and the second sequence of blood samplings was conducted. This Latin square experiment was repeated for each of the four pairs of pigs. The BW of the eight pigs when they were fed the 16% CP diet and the 12% CP + AA diet were 48.5 ± 1.8 and 48.4 ± 1.4 kg, respectively.

The net portal absorption of lysine or threonine was calculated by multiplying the porto-arterial plasma concentration difference of the amino acid by the portal plasma flow rate. The portal vein plasma flow rate (**PVPF**) was estimated by the indicator-dilution technique, using PAH as the indicator (Roe et al., 1966; Yen and Pond, 1990). The PVPF was calculated with the following equation: $PVPF = Ci \times IR \times (PAHpv - PAHa)^{-1}$; where Ci is the concentration of PAH infusion solution (mg/mL), IR is infusion rate (mL/min), and PAHpv and PAHa are portal and arterial PAH concentrations (mg/mL), respectively.

Data from the four 2 × 2 Latin squares were subjected to repeated-measures analyses, using the mixed model procedure of SAS (SAS Inst., Inc., Cary, NC) as recommended by Littell et al. (1998). The model included diet, time of blood sampling (**time**), and diet × time. Square, animal, and test sequence were considered to be random effects. Autoregressive order 1 was used for covariance structure. The pig was the experimental unit. The area under the curve of the absorbed AA was obtained by a trapezoidal technique. The dietary effect on the area under the curve was tested by ANOVA, using the GLM procedure of SAS. An alpha level of 0.05 was used for determination of statistical significance of differences among treatments, with a trend reported at $P < 0.15$.

Results

The portal and arterial plasma lysine concentrations of pigs are presented in Figure 1. For both the portal and arterial plasma lysine concentrations, there was a diet × time interaction ($P < 0.01$). Feeding increased ($P < 0.01$) concentrations of plasma lysine in both portal and arterial samples throughout the 6-h postprandial period. Both the portal and arterial plasma lysine concentrations were greater ($P < 0.01$) when pigs were given the 12% CP + AA diet than when given the 16%

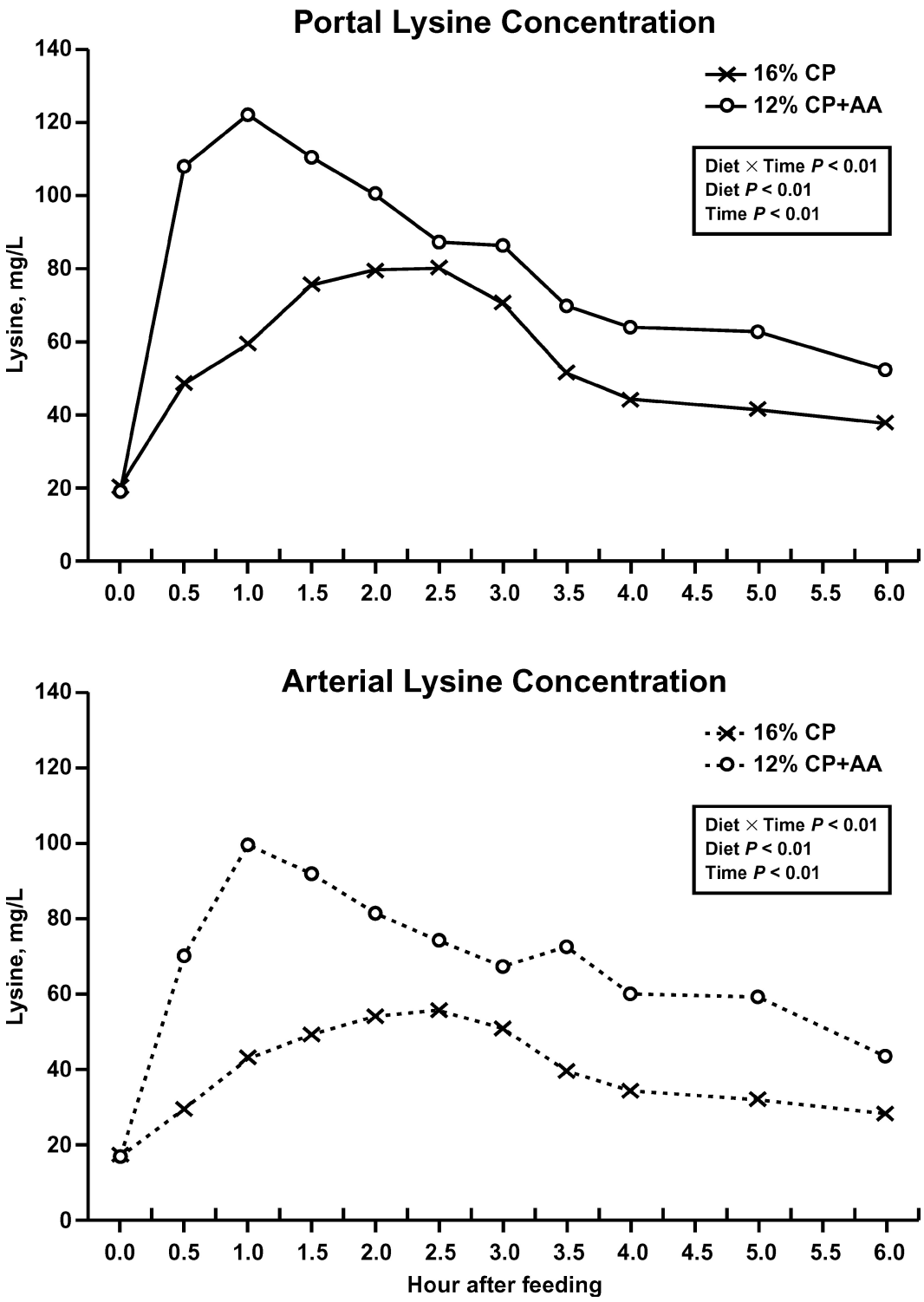

Figure 1. Portal and arterial plasma concentrations of lysine in pigs. Values are means for eight pigs (48.5 ± 1.6 kg BW).

CP diet. When pigs were fed the 12% CP + AA diet, the portal and arterial plasma lysine concentrations attained their maximal levels at 1 h postprandial, whereas the peak level of the elevated portal and arterial plasma lysine concentrations was reached at 2.5 h postprandial when feeding the 16% CP diet.

Figure 2 shows the portal and arterial plasma threonine concentrations of pigs. Similar to plasma lysine concentration, there was a diet × time interaction ($P < 0.01$) for the portal and arterial plasma threonine concentrations. Both portal and arterial plasma threonine concentrations of pigs increased ($P < 0.01$) after

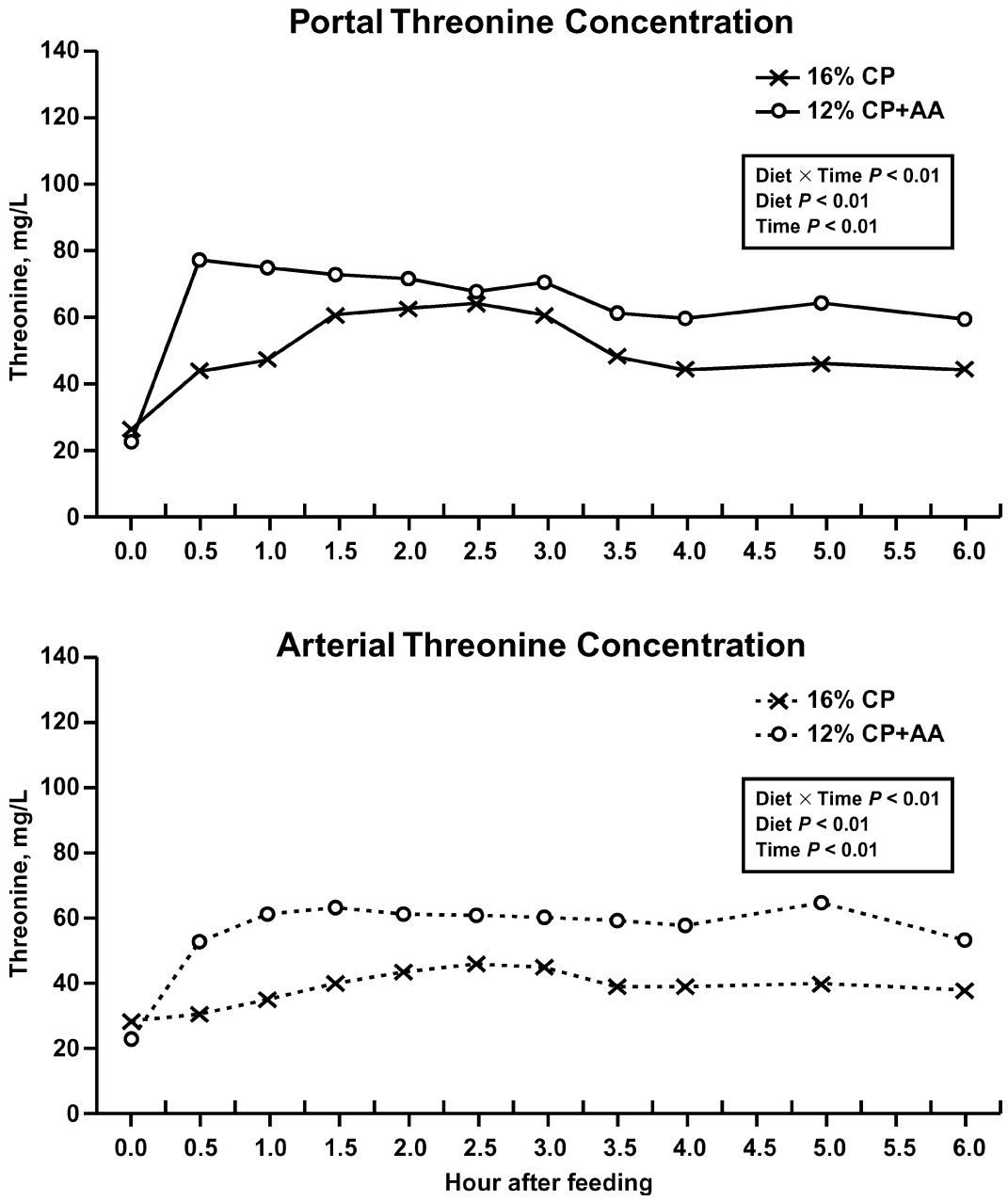

Figure 2. Portal and arterial plasma concentrations of threonine in pigs. Values are means for eight pigs (48.5 ± 1.6 kg BW).

feeding. Again, the portal and arterial plasma threonine concentrations were greater ($P < 0.01$) when pigs were fed the 12% CP + AA diet vs. the 16% CP diet. When the 12% CP + AA diet was given to pigs, the portal plasma threonine concentration attained its peak at 0.5 h postprandial. With the feeding of the 16% CP diet, the portal plasma threonine concentration reached the maximum level at 2.5 h postprandial. In regard to the arterial plasma threonine concentration, it elevated to the maximum level at 1.0 h postprandial when pigs were fed the 12% CP + AA diet, but at 2.5 h postprandial when the 16% CP diet was given to pigs.

The portal vein plasma flow rate of pigs is illustrated in Figure 3. There was no diet × time interaction ($P = 0.87$) affecting PVPF of pigs. However, the PVPF was influenced by time and diet ($P < 0.01$). After feeding, the PVPF increased ($P < 0.01$). The elevated PVPF was maintained during the 6-h postprandial period. The PVPF of pigs was less ($P < 0.01$) when they were fed the 12% CP + AA diet than the 16% CP diet.

Figure 4 depicts the net portal absorption of lysine and threonine. Once again, there were diet × time interactions ($P < 0.05$). As shown in the upper panel of Figure 4, when the 12% CP + AA diet was given, the net portal

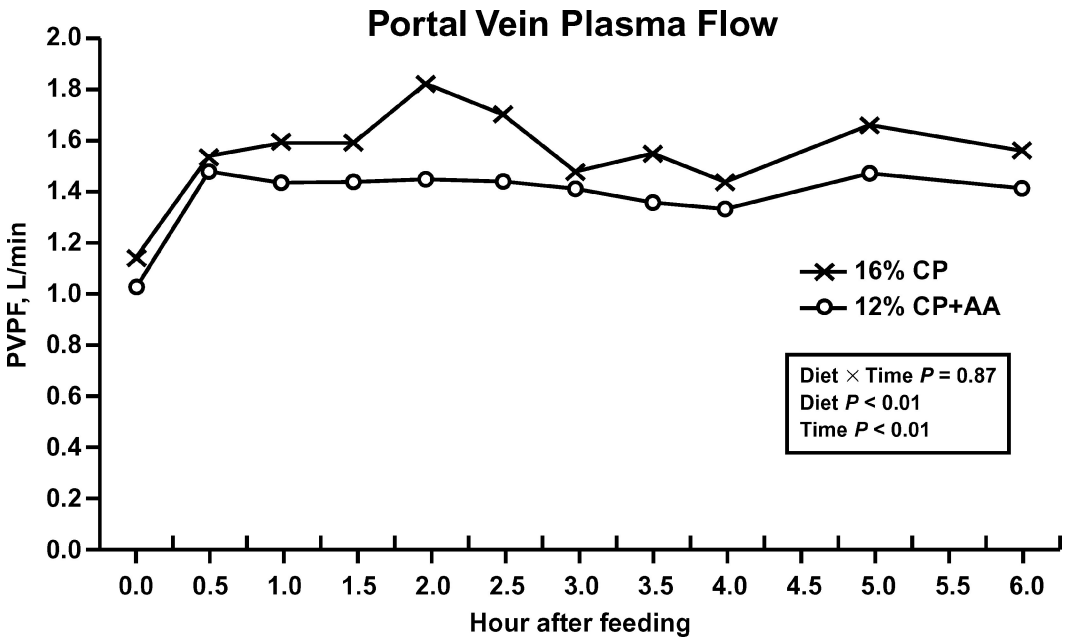

Figure 3. Portal vein plasma flow (PVPF) rates of pigs. Values are means for eight pigs (48.5 ± 1.6 kg BW).

absorption of lysine reached the peak at 0.5 h postprandial, whereas with the feeding of the 16% CP diet, the lysine absorption of pigs continued to increase after feeding, attained its peak at 2.5 h postprandial. The overall net portal absorption of lysine during the 6-h postprandial period tended to be less ($P = 0.06$) from feeding the 12% CP + AA diet than the 16% CP diet.

The pattern of threonine absorption in pigs (lower panel of Figure 4) was similar to that of lysine absorption and was influenced ($P < 0.05$) by time × diet interaction. The peak threonine absorption was also reached at 0.5 h postprandial when the 12% CP + AA diet was fed, but at 2.5 h postprandial when the 16% CP diet was given. The overall net portal absorption of threonine during the 6-h postprandial period tended to be less ($P = 0.09$) when pigs were fed the 12% CP + AA diet than the 16% CP diet.

As shown in Figure 5, there was no diet × time interaction for the portal ($P = 0.30$) and arterial ($P = 0.21$) plasma concentrations of methionine in pigs. After feeding, the concentrations of portal and arterial plasma methionine increased ($P < 0.01$). The concentrations were less ($P < 0.01$) in pigs when they were fed the 12% CP + AA diet compared with the 16% CP diet. No diet × time interaction ($P = 0.39$) could be detected for the net portal absorption of methionine (Figure 6). The portal methionine absorption increased ($P < 0.01$) after feeding, and the increase was less ($P < 0.01$) when pigs were fed the 12% CP + AA diet than the 16% CP diet. The responses in pig's net portal absorption of arginine, histidine, isoleucine, leucine, phenylalanine, and valine (Figures 6 and 7) and plasma concentrations of these AA (data not shown) to the two test diets were similar to the methionine responses.

Table 3 shows the total quantity of net portal AA absorption over the 6-h postprandial period as calculated as the area under the curve of net portal AA absorption. Compared with the 16% CP diet, feeding the 12% CP + AA diet tended to result in less total quantity of absorbed lysine ($P = 0.13$) and threonine ($P = 0.10$) over the 6-h postprandial period. However, feeding the 12% CP + AA diet instead of the 16% CP diet did produce less ($P < 0.05$) 6-h total absorption of methionine, arginine, histidine, isoleucine, leucine, phenylalanine, and valine. Values of the fractional absorption, calculated as the proportion of dietary AA intake that was absorbed over the 6-h postprandial period, are also presented in Table 3. The fractional absorption values of lysine and threonine tended to be less ($P = 0.12$ and $P = 0.09$, respectively) for the 12% CP + AA diet than the 16% CP diet. When pigs were fed the 12% CP + AA diet, values of their fractional absorption of arginine, histidine, isoleucine, leucine, phenylalanine, and valine were also tended to be less ($P \leq 0.11$) compared with the 16% CP diet. No fractional absorption of methionine intake was estimated because no determination was conducted on dietary methionine concentration.

Discussion

The 16% CP diet of the present study was formulated to contain 0.83% protein-bound total lysine and 0.67% protein-bound total threonine. The 12% CP + AA diet was calculated to contain 0.54% protein-bound lysine and 0.51% protein-bound threonine. The 12% CP + AA diet was also supplemented with 0.29% crystalline lysine (0.35% lysine hydrochloride) and 0.16% crystalline

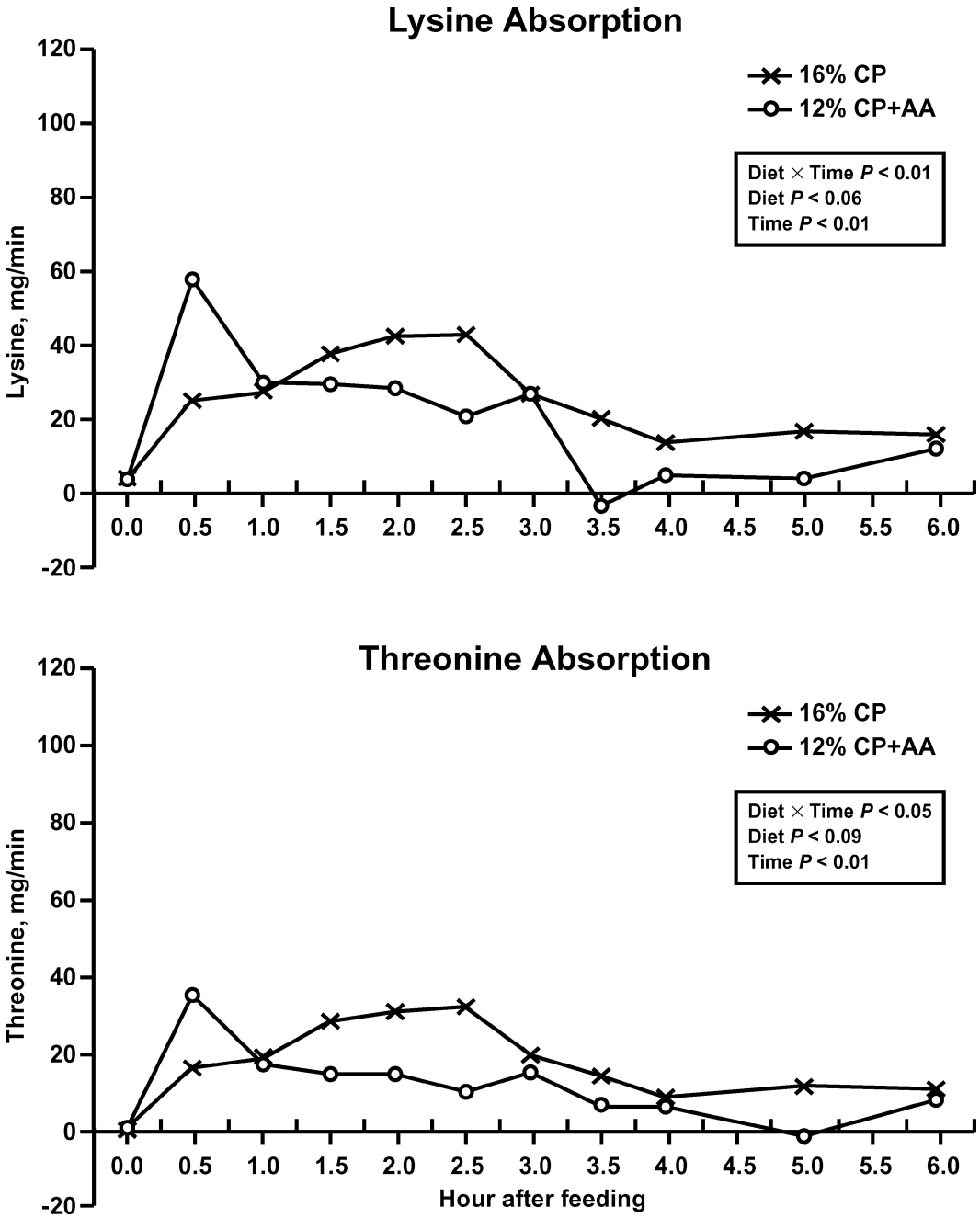

Figure 4. Net portal absorption of lysine and threonine in pigs. Values are means for eight pigs (48.5 ± 1.6 kg BW).

threonine, so its total lysine and total threonine concentrations were 0.83% and 0.67%, respectively. The analyzed values of total lysine were 0.82 and 0.83%, and those of total threonine were 0.62 and 0.63% for the 16% CP diet and the 12% CP + AA diet, respectively (Table 2). With once daily feeding of 1.2 kg of feed, pigs fed either test diet received a daily intake of 9.96 g of total lysine (0.83% analyzed lysine × 1,200 g of feed) and 7.56 g of total threonine (0.63% analyzed threonine × 1,200 g). Although pigs fed the two test diets ingested the same 9.96 g of total lysine and 7.56 g of total threonine, the peak portal absorption of both lysine and threonine in pigs occurred at 0.5 h postprandial when the 12% CP + AA diet was fed, but at 2.5 h postprandial when the 16% CP diet was given. This early appearance of portal-absorbed lysine and threonine from feeding the 12% CP + AA diet indicates a rapid absorption of the supplemental crystalline lysine and threonine from the 12% CP + AA diet compared with the protein-bound lysine and threonine from the 16% CP diet.

After the crystalline lysine and threonine were rapidly absorbed by the first postprandial hour, the 12% CP + AA diet would have less total lysine and threonine available from the protein-bound lysine and threonine than the 16% CP diet during the remaining postprandial period. This lesser availability of protein-bound

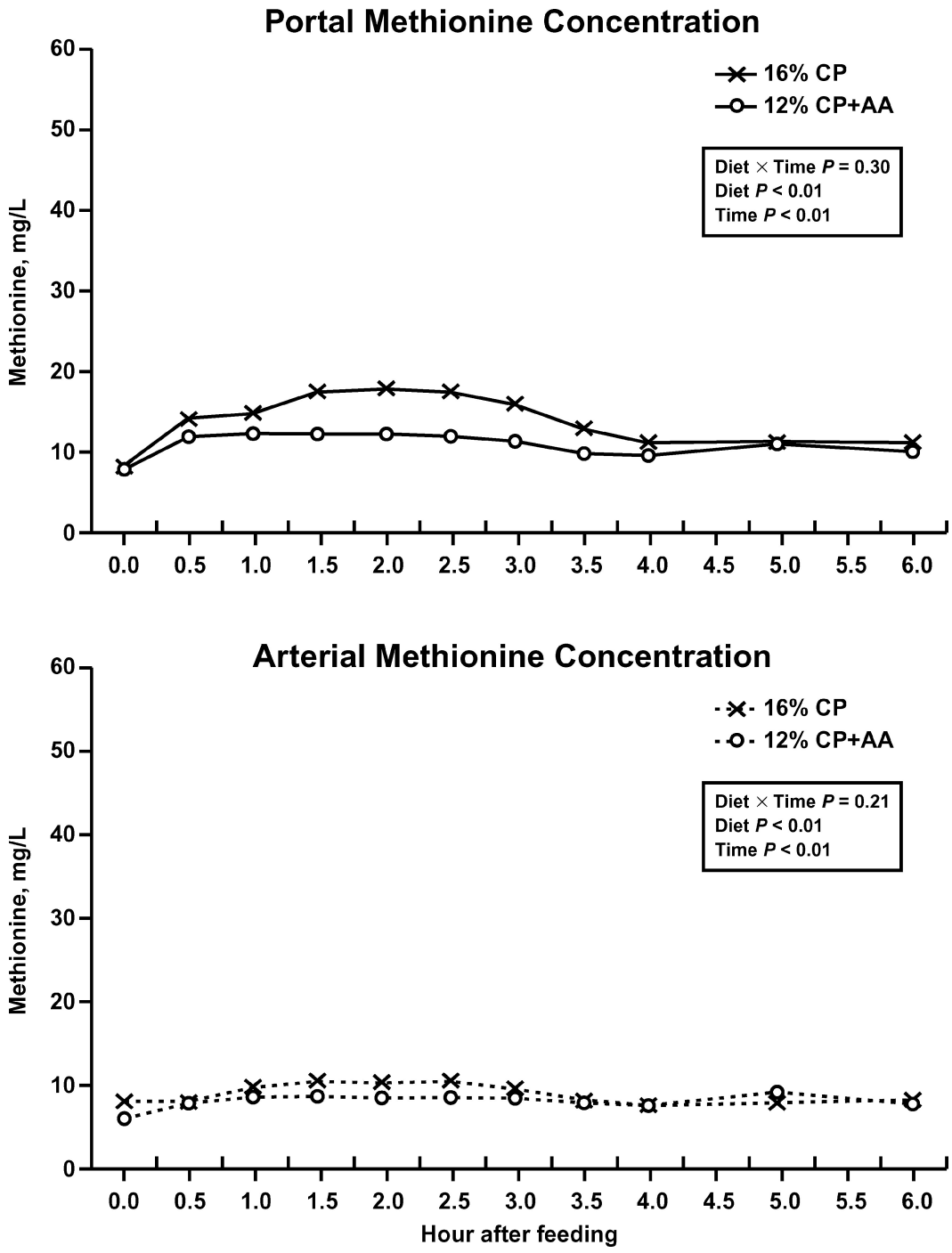

Figure 5. Portal and arterial plasma concentrations of methionine in pigs. Values are means for eight pigs (48.5 ± 1.6 kg BW).

lysine and threonine in the 12% CP + AA diet is well reflected in its lesser net portal absorption of both lysine and threonine during the 1 to 6 h postprandial period. Although pigs were fed the same amounts of total lysine and total threonine, the overall 6-h postprandial portal absorptions of lysine and threonine tended to be less when pigs were fed the 12% CP + AA diet rather than the 16% CP diet. The tendency of greater portal absorptions of lysine and threonine when pigs were given the 16% CP diet might be caused by increased reabsorption of endogenous lysine and threonine from greater gut endogenous protein secretion for hydrolysis and release of dietary protein-bound AA. Between 70 and 80% of endogenously secreted protein is digested along with dietary protein and absorbed before the terminal ileum in pigs (Nyachoti et al., 1997). According to Boisen and Moughan (1996), the ileal endogenous protein of growing pigs contains high concentrations of threonine (4.5%) and lysine (3.0%). Ample evidence has also shown that the quantity of endogenous protein secreted

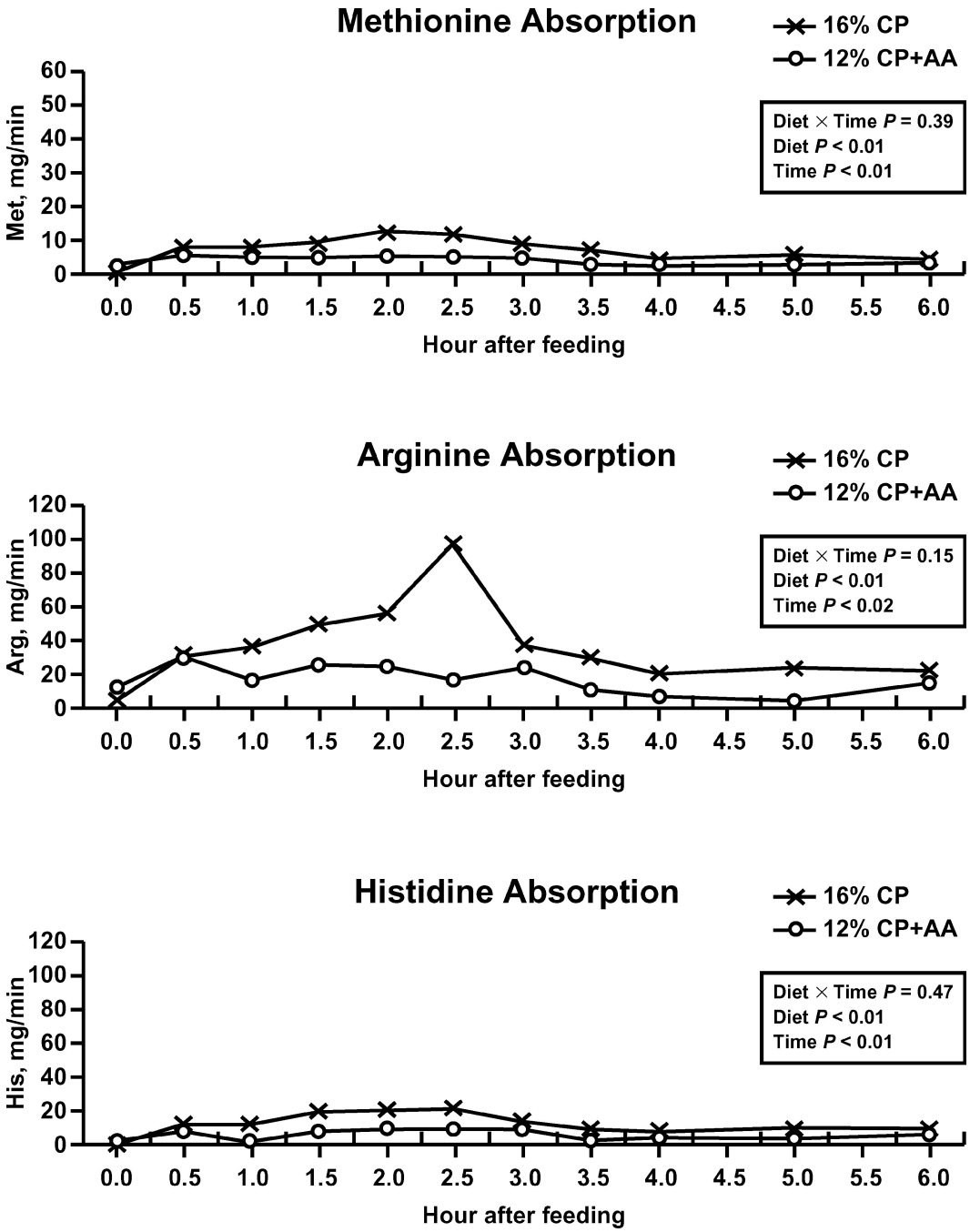

Figure 6. Net portal absorption of methionine (Met), arginine (Arg), and histidine (His) in pigs. Values are means for eight pigs (48.5 ± 1.6 kg BW).

into the gut increases substantially with increasing dietary protein levels (Nyachoti et al., 1997). With more dietary protein in the 16% CP diet than in the 12% CP + AA diet, more endogenous gut protein might have been secreted, and thus, more endogenous threonine and lysine probably were reabsorbed along with released dietary protein-bound AA when pigs were fed the 16% CP diet.

In the present study, no direct measurement of endogenous protein secretion was conducted. Thus, no clear evidence is available to support the contention that the increased net portal absorption of threonine and lysine from feeding the 16% CP diet might result from an increased endogenous AA secretion. However, an increase in endogenous secretion and reabsorption of threonine and lysine when the 16% CP diet was fed may be inferred from the results in PVPF of the present study. With the feeding of the 16% CP diet, the PVPF of pigs was greater than that of the 12% CP + AA diet. This greater PVPF could be a hemato-circulatory response to a more active gastrointestinal function, which might relate to secretion and reabsorption of endogenous AA associated with digestion of the 16% CP diet to release more protein-bound AA. Nevertheless,

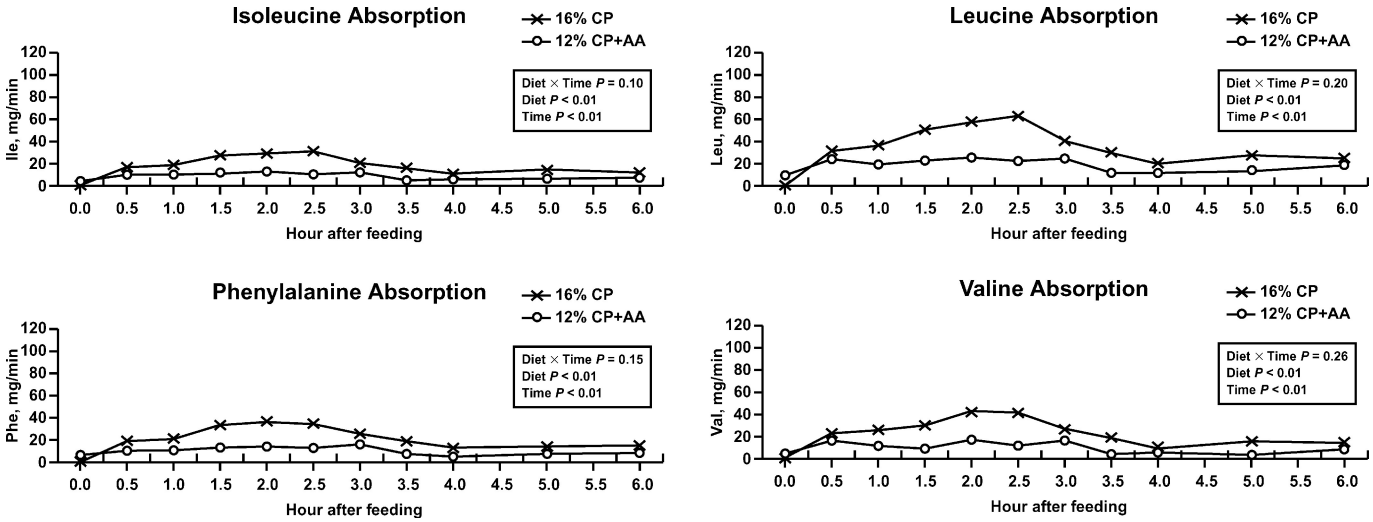

Figure 7. Net portal absorption of isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and valine (Val) in pigs. Values are means for eight pigs (48.5 ± 1.6 kg BW).

further studies are needed to unequivocally illustrate the relationship between endogenous AA secretion and net portal AA absorption.

The rapid absorption of crystalline AA during the early postprandial period from feeding the 12% CP + AA diet could result in lower utilization efficiency of absorbed crystalline AA for protein accretion and body growth in pigs fed once daily. This contention on utilization efficiency, particularly for growth performance, however, could not be proved or disproved in the present study because of the short feeding period of test diets (only one meal of test diets in the current study). With a longer feeding period (>5 d), inefficient utilization of supplemental crystalline lysine for growth performance and N retention in once-daily feeding pigs has been reported by Batterham and Murison (1981) and Cook et al. (1983). Poor utilization of crystalline threonine in pigs fed once daily had also been observed (Cole, 1992).

Batterham and Bayley (1989) proposed that the inefficiency of crystalline lysine utilization in pigs fed once daily was caused by the rapid absorption of crystalline lysine relative to other protein-bound AA and the resulting temporary AA imbalance at the sites of protein synthesis. The direct measurements of net portal absorption of lysine and threonine in the present study demonstrate that crystalline lysine and threonine indeed are absorbed more rapidly than protein-bound lysine and threonine during the first postprandial hour in growing pigs fed once daily. The similarity of absorption patterns for crystalline lysine and crystalline threonine observed in the present study suggests that rapid absorption of crystalline AA compared with protein-bound AA is a general phenomenon in pigs. This contention of rapid absorption of crystalline AA would gain more support if the absorption of AA other than lysine and threonine were also determined in the present study. Originally, we did plan to demonstrate that crystalline tryptophan was absorbed more quickly than protein-bound tryptophan. Therefore, in addition to lysine and threonine, crystalline tryptophan was also added to the

Table 3. Total net portal absorption of amino acids over the 6-h postprandial period, dietary amino acid intake, and fractional absorption of amino acid intake in pigs[a]

	Total net portal AA absorption, g[b]			Dietary intake, g			Fractional absorption of AA intake, %		
Item	16% CP	12% CP + AA	*P*-value	16% CP	12% CP + AA	*P*-value	16% CP	12% CP + AA	*P*-value
Lysine	8.6	6.9	0.13	9.9	9.9	0.99	87	70	0.12
Threonine	6.2	4.2	0.10	7.5	7.5	0.99	83	57	0.09
Arginine	12.8	6.1	0.02	12.5	8.9	0.01	103	69	0.09
Histidine	4.5	2.4	0.03	5.4	4.6	0.01	84	53	0.07
Isoleucine	6.2	3.0	0.02	8.3	6.2	0.01	75	48	0.06
Leucine	12.8	7.1	0.03	18.8	15.8	0.01	68	45	0.10
Methionine	2.5	1.3	0.05	—	—		—	—	
Phenylalanine	7.6	4.0	0.02	9.7	7.6	0.01	78	53	0.10
Valine	8.0	3.9	0.03	9.8	7.4	0.01	82	53	0.11

[a]Values for total and fractional absorption are means of eight pigs fed once daily 1.2 kg of feed.
[b]Estimated from the area under the curve of absorbed AA over 6-h postprandial period.

12% CP + AA diet in the present study. Unfortunately, tryptophan was accidentally omitted from the standard solution for AA analysis. Therefore, no determination could be conducted for plasma tryptophan concentrations in portal and arterial samples, as well as net portal absorption of tryptophan. Nevertheless, rapid absorption of crystalline tryptophan and the resulting AA imbalance could be the reason why Sawadogo et al. (1997) observed that the utilization efficiency of crystalline tryptophan was only 25% of that for protein-bound tryptophan in early-weaned pigs (4 kg of BW) force-fed three times daily.

Whereas AA imbalance could be a reason for the inefficiency of utilization of rapidly absorbed crystalline AA, accelerated oxidation of rapidly absorbed AA might also reduce their efficiency of utilization. Boirie et al. (1997) observed a rapid postprandial increase in plasma leucine concentration in young healthy human adults ingesting a single meal of ^{13}C-leucine labeled whey protein instead of ^{13}C-leucine labeled casein. The protein accretion over the 7-h postprandial period was less when the whey protein rather than the casein was fed. The decreased protein accretion from feeding the whey protein was associated with an increased oxidation of rapidly absorbed ^{13}C-leucine. In pigs fed once daily a diet supplemented with crystalline AA, it is possible that the rapidly absorbed crystalline AA are oxidized more quickly, resulting in a decrease in the utilization efficiency of supplemental crystalline AA.

The present study illustrates clearly that crystalline AA were absorbed more rapidly than protein-bound AA. The study, however, provides no information regarding where the rapid absorption of crystalline AA took place in the gastrointestinal (**GI**) tract. Because the hepatic portal vein of pigs collects blood from the stomach, small intestine, large intestine, pancreas, and spleen, the AA absorbed into the portal vein are a combined product of the entire GI tract and not from a specific GI segment. Yet, the rapid absorption of crystalline AA in pigs most likely occurs in the small intestine and not in the stomach or the large intestine. As pointed out by Yen (2001), there is no evidence in the literature for AA absorption in the stomach of pigs. The conclusion is based on the findings of Low (1989), who 1) detected no ^{14}C in the peripheral blood following administration of ^{14}C-labeled AA into the stomach of anesthetized pigs with ligated esophageal and pyloric sphincters, and 2) demonstrated no active transport of AA in isolated gastric mucosa in vitro. With barrows weighed (44 kg) similar to the present study (48.5 kg) and fitted with postvalvular T-cecum cannula, Buraczewska and Swiech (2000) showed a complete disappearance of supplemental crystalline threonine from the lumen of the small intestine. These results confirm that the small intestine rather than the large intestine is the site of rapid absorption of crystalline AA in pigs.

In the present study, with equal dietary lysine and threonine intake for the treatments, a tendency of less fractional absorption for both lysine and threonine intake was detected for the 12% CP + AA treatment compared with the 16% CP treatment. This tendency reflects differences between the two treatments in their total quantities of lysine and threonine absorption over the 6-h postprandial period. The fractional absorption values of lysine and threonine intake when pigs were fed the 12% CP + AA diet were 70% and 57%, respectively. With the feeding of the 16% CP diet, fractional absorption values for the lysine and threonine intake were 87% and 83%, respectively. These fractional absorption values most likely were overestimated, because a portion of totally absorbed lysine and threonine were not from dietary intake but were reabsorbed AA from endogenous secretions. Whereas total net absorption of six other indispensable AA were significantly less for the 12% CP + AA diet than the 16% CP diet in the present study, there was only a tendency of difference between the two diets in their fractional absorption of dietary intake. Nevertheless, the values of fractional absorption for these AA, except arginine, would be overestimated, as was the case for lysine and threonine. Again, reabsorption of these AA from endogenous secretion is the reason for the overestimation. In addition to lysine and threonine, the protein of pigs' ileal endogenous secretion also contains 1.5% histidine, 2.5% isoleucine, 4.0% leucine, 3.0% phenylalanine, and 3.5% valine (Boisen and Moughan, 1996). When feeding of the 16% CP diet, the fractional absorption for dietary arginine was 103%. This greater than 100% fractional absorption for arginine probably resulted from net arginine synthesis in the intestine as found in neonatal pigs (Burrin and Stoll, 2003).

Besides differences in endogenous protein secretion and AA reabsorption, other factor(s) might also produce the dietary effect on the portal absorption of lysine and threonine observed in the present study. When expressed as a percentage of the 16% CP diet, the reduction in the fractional absorption of AA intake from the 12% CP + AA diet amounted to 20% ([87 – 70]/87) for lysine and 31% ([83 – 57]/83) for threonine, and ranged from 32 to 37% for six other AA. The lower reduction in the fractional absorption for lysine intake implies a lesser extent in the gut metabolism or a greater efficiency in the portal absorption of dietary lysine than for threonine and other AA. This reasoning, however, contradicts the findings of other studies. In young pigs fed a single bolus liquid milk replacer, Burrin and Stoll (2003) measured portal absorption for 8 h and found that gut metabolism was greater and the portal absorption of dietary intake was less for lysine than for threonine and other AA. Nonetheless, results of the present study would suggest that factor(s) associated with gut metabolism and portal absorption efficiency of AA could be another cause for differences between the 16% CP and the 12% CP + AA diets in their portal absorption and fractional absorption of dietary lysine and threonine. Additional research is required to fully delineate the underlying factor(s) that yield(s) dietary differences in the portal absorption of AA.

The 12% CP + AA diet of the present study was formulated and analyzed to contain less methionine, arginine, histidine, isoleucine, leucine, phenylaline, and valine than the 16% CP diet (Table 2). With these differences in the dietary content, it is not surprising that the net portal absorption of these AA during the 6-h postprandial period was less when pigs were fed the 12% CP + AA diet vs. the 16% CP diet. Because no crystalline form of these AA was added to the test diets in the present study, the plasma concentration and the net portal absorption of these AA were related directly to the protein-bound AA. No diet × time interaction was detected for the patterns of the plasma concentration and the net portal absorption of these AA during the 6-h postprandial period.

Implications

Lowering dietary protein level and supplementing crystalline amino acids decrease nitrogen excretion and environmental pollution; however, crystalline amino acids are absorbed more rapidly than protein-bound amino acids in pigs. This can cause a temporary surplus of absorbed crystalline amino acids and result in an imbalance of amino acids at the sites of protein synthesis when a diet supplemented with crystalline amino acids is fed only once daily. The rapidly absorbed crystalline amino acids may also be oxidized too quickly for efficient protein accretion. Although most pigs are generally allowed ad libitum access to feed, inefficient utilization of supplemental crystalline amino acids resulting from once-daily feeding is a concern in gestating sows and pigs that are restricted-fed in many research studies.

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